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Letter to the Editor

Langerhans cells in the sebaceous gland of the murine skin

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Background

Langerhans cells (LCs) are professional antigen-presenting cells residing in the skin of mammals (1). They are the epidermal variant of dendritic cells (DCs) and have a strong immunogenic capacity *in vitro*. They strongly express MHC class II and the C-type lectin Langerin/CD207. In uninflamed skin, LCs lack expression of maturation markers such as CD86. The epidermal frequency of LCs is well established in different mouse strains (2–4). However, little is known about the occurrence of LCs in epidermal appendages. This is particularly important considering that micro-organisms make use of these voids to deeply invade the skin (5). In addition, hair follicles were recently described being portals of entry for LC precursors in response to stress (6). Whereas LCs do occur in the infundibular epidermis of the hair follicle (S1), their relation to sebaceous glands, which were mainly studied in humans (7; S2–S5), has not yet been elucidated in mice.

Questions addressed

We aimed at characterizing and quantifying epidermal LCs associated with the sebaceous glands visible in dermal sheets. We focused on BALB/c and C57BL/6 mice, which are frequently employed in immunological studies and exhibit distinct repartitions and phenotypes of DC subsets (3,4) (S9,S10).

Experimental design

Upon cleavage of the dermo-epidermal junction of dorsal ear skin of BALB/c and C57BL/6 mice, dermal sheets were separated and stained using antibodies against MHC class II, Langerin/CD207 and CD86. Unspecific binding of fluorochrome-coupled antibodies or lipophilic staining by Nile Red allowed visualization of the sebaceous glands. Sebaceous glands as well as sebaceous gland-associated immunolabelled cells were analysed and counted (Data S1).

Results

Langerhans cells are associated with sebaceous glands

Sebaceous glands could be clearly identified by Nile Red staining (Fig. S1A) and were also very susceptible to unspecific binding of fluorochrome-coupled antibodies (Fig. S1B) to the lipidic content of the glands. We consistently found sebaceous gland-associated LCs expressing MHCII and Langerin (Fig. 1a and b) but devoid of CD86, a marker of DC maturation (Fig. 1c). Tridimensional reconstruction confirmed that LCs were directly interacting with sebocytes (Videos S1A and B).

Higher frequency of sebaceous gland-associated Langerhans cells in BALB/c mice

We used confocal microscopy to quantify LCs within sebaceous glands in Nile Red/Langerin double stainings. Whereas sebaceous glands appeared with an identical density (~65/mm^2) in both mouse strains (Fig. 2a), they contained LCs much more frequently in BALB/c mice (69.5% ± 2.8; mean ± SEM) than in C57BL/6 mice (18.3% ± 1.8) (Fig. 2b). This represents 46.5 sebaceous gland-associated LCs per mm^2 in BALB/c and 12.3 per mm^2 in C57BL/6 mice.

Sebaceous gland-associated Langerhans cells represent a substantial part of Langerin+ cells observed in dermal sheets

The dermis contains several resident DC populations, one of which expresses Langerin at levels similar to LCs (1; S7,S8). To compare the relative importance of Langerin+ dermal DCs and sebaceous gland-associated, epidermal LCs, we enumerated all Langerin+ cells visible in dermal sheets. We validated our stainings by counting LCs in epidermal sheets. As expected (1,4), epidermal LCs had a density of 830 cells/mm^2 in BALB/c mice and 522 cells/mm^2 in C57BL/6 mice. In dermal sheet preparations from BALB/c and C57BL/6, Langerin+ cells (including gland-associated LCs) occurred at a density of 100 cells/mm^2 and 76 cells/mm^2, respectively (Fig. S3). Thus, gland-associated LCs account for 46.5% of all Langerin+ cells in dermal sheets of BALB/c mice and 16.2% in C57BL/6.

Conclusions

We demonstrate a tight association of LCs with sebaceous glands of the murine skin. Langerin+ DCs observed within or isolated from steady-state dermis used to be considered as migrating LCs (S6), although some of them did not display the typical morphology and phenotype (e.g. CD86 expression) of mature and migrating DCs. However, immature resident Langerin+ dermal DCs have been identified (1; S7,S8). The cells we describe here are situated inside the sebaceous glands, which are visible only on dermal sheets upon separation from the epidermis, but are delineated by

Experimental Dermatology

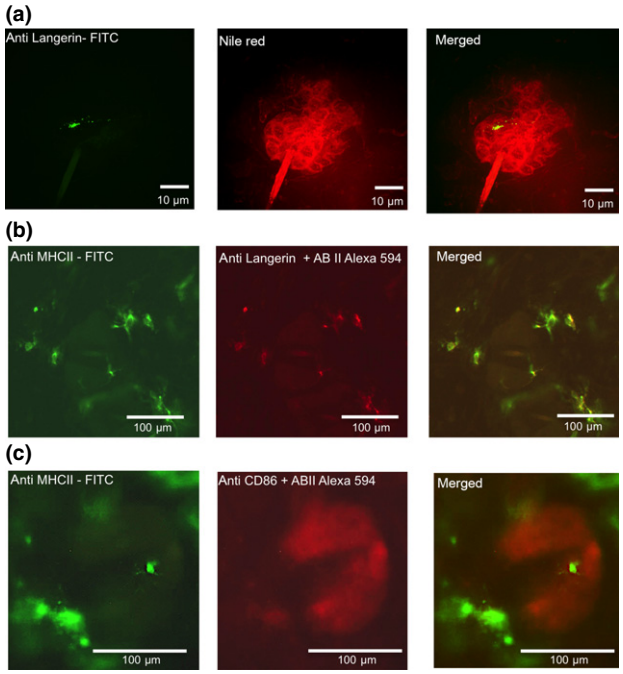

Figure 1. Immature Langerhans cells are associated with sebaceous glands. Dermal sheets from the dorsal ear skin of BALB/c mice were stained with (a) anti-Langerin (green) and Nile Red lipophilic stain (red) and analysed by confocal microscopy. Alternatively, dermal sheets were observed by epifluorescence microscopy after staining with (b) anti-MHCII (green) and anti-Langerin (red) or (c) anti-MHCII (green) and anti-CD86 (red). Images are representative of >80 fields of view.

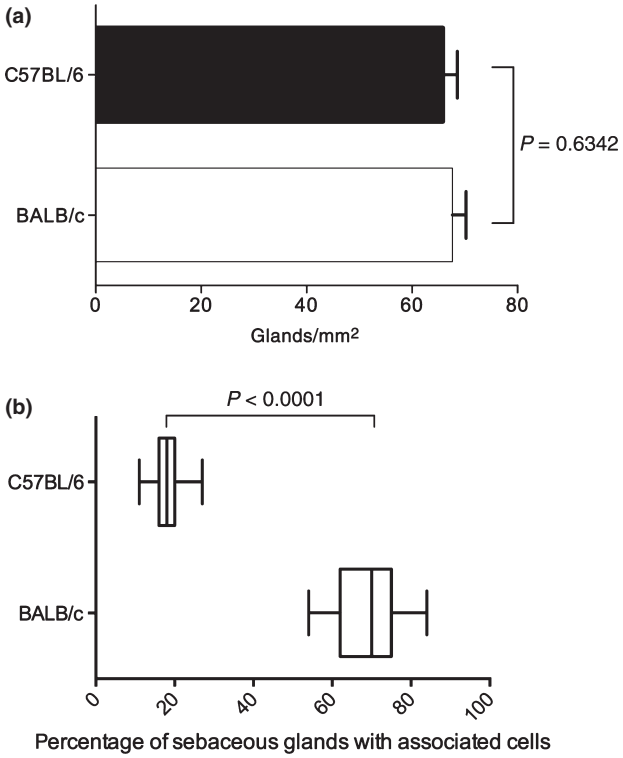

Figure 2. BALB/c mice have more sebaceous gland-associated Langerhans cells than C57BL/6 mice. (a) The frequency of sebaceous glands is similar in both mouse strains. Mean values were calculated from >20 sheets visualized by epifluorescence microscopy. Error bars represent SEM. (b) Sebaceous gland-associated LCs are more commonly found in BALB/c mice. Gland-associated MHCII+ or Langerin+ cells were counted in dermal sheets counterstained with Nile Red. Box-and-whiskers plot was calculated from confocal microscopy analyses of 11 BALB/c and 7 C57BL/6 mice, accounting for 401 and 321 sebaceous glands, respectively.

epithelial cells and therefore classified as epidermal appendages. Consequently, sebaceous gland-associated DCs should be qualified as LCs. Intriguingly, gland-associated LCs were more frequent in BALB/c mice, in line with their particularly dense epidermal LC network (2) and reminiscent of other strain-related variations in DC phenotypes and distribution patterns (3,4; S9,S10).

Dermal DCs are usually studied after removal of the epidermis and digestion of the dermis. Depending on the reagents/enzymes chosen and the separation procedure (S11,S12), this technique is prone to some contamination of the resulting dermal suspension by cells actually originating from epidermal appendages, which extend deeply into the dermis. Most Langerin+ dermal DCs express CD103 (S13), as opposed to LCs. Nevertheless, relatively rare Langerin+ CD103- dermal DCs have been described (8). Although this study made use of an elegant bone marrow transfer system, it remains possible that sebaceous gland-associated LCs, which represent up to 46.5% of all Langerin+ cells in dermal sheets, may account for a part of Langerin+ CD103- dermal DCs isolated from undisturbed mice.

In line with our previous results (4), the density of Langerin+ dermal DCs (i.e. Langerin+ cells not associated with sebaceous glands in dermal sheets) was relatively similar in BALB/c and C57BL/6 mice (53.5 and 63.7/mm², respectively). Thus, the small but significant strain-dependent difference between BALB/c and C57BL/6 for total Langerin+ cells visible in dermal sheets can be mostly attributed to a higher frequency of gland-associated LCs (46.5 and 12.3/mm², respectively). We conclude that gland-associated LCs should be taken into account in future studies on DC subsets isolated from the dermis.

Commensal bacteria inhabit sebaceous glands as well as the infundibular regions of the pilosebaceous unit, where gland-associated LCs might prohibit inadequate responses to steady-state skin antigens (2). Human and murine LCs are equipped with CD1 molecules, which might be used for presentation to NKT cells of lipidic self-antigens from sebum (9). The location of gland-associated LCs suggests a role in immune responses involving sebaceous glands, the most frequently associated pathology being acne vulgaris. Therefore, although specifically studying sebaceous glands remains challenging (7) (S14), functional investigations on LCs from this location could be of fundamental and clinical value, especially in the context of cutaneous inflammation.

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Contributions

B.H. and D.E.S. performed experimental work under the supervision of C.H.T. and P.S., respectively. M.H. analysed the data. B.H., N.R. and V.F. designed the research study, analysed the data and wrote the manuscript.

Conflict of interest

The authors have declared no conflicting interests

Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Figure S1. Sebaceous glands can be detected in dermal sheets.

Figure S2. Langerhans cells are located within sebaceous glands.

Figure S3. Frequency of total Langerin+ cells in dermal and epidermal sheets of two mouse strains.

Video S1. Three-dimensional reconstruction of a sebaceous gland stained with Nile Red, The video, including a Langerin positive LC (green), was compiled from confocal microscopy Z-stack imaging.

Video S2. Three-dimensional reconstruction of a sebaceous gland stained by unspecific binding of the FITC-conjugated secondary antibody.

Data S1. Materials and Methods.

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